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REMARKS

THE INVENTION:

This invention is a novel means for selectively controlling the expression of a target protein by binding a sequence specific oligonucleotide to a subsequence of the target protein coding region of a mRNA.

It is an invention that reflected thinking in direct conflict with the conventional wisdom of the time. Although others were discussing the possibility of using viral nucleic acids and mRNA as targets for control of expression using complementary oligonucleotides, control of mRNA by targeting the coding region was not suggested. A detailed review of the literature explains why. Those of skill expected the extensive secondary structure of mRNA to prevent duplexing between the mRNA and oligonucelotides except in the noncoding regions of the mRNA which are free of secondary structure and are where ribosomes initially bind.

In reality and surprisingly, the secondary structure of mRNA coding regions was not a problem - at least *in vivo*. The real problem was the length of the oligonucleotides used to arrest translation. In the early studies, trimers were used to arrest translation and there was no evidence that these oligonucleotides arrested translation *in vivo* or *in vitro* when directed to the coding regions of mRNA. It was ultimately demonstrated that such short oligonucleotides are simply too short to arrest translation when attempting to halt ribosomal elongation on a mRNA. In contrast, these short species are adequate to arrest translation only if binding to tRNA anticodons or the initiation sites of mRNA.

The literature provided with this Amendment explains that the conventional wisdom taught that secondary structure was the problem. The oligonucleotides of the prior art were not arresting translation *in vivo* because they did not have access to the coding region of mRNA. The subject application addresses this defective reasoning by expressly articulating that the proper length oligonucleotides were needed and that secondary structure was not a problem to successful arrest of translation.

THE STATUS OF THE CLAIMS:

Claims 53-61 and 63 were pending. Claims 53-61 and 63 are cancelled and claims 64-71 are added.

The amendments to the claims are not considered to be new matter. Independent claim 64 derives support from original claim 20 and claim 63. The proviso language is not considered to be new matter. The remaining changes are minor textual changes. These changes are grammatical and do not introduce new matter. Claim 65 encompasses oligodeoxynucleotides that consist of a base sequence which is entirely complementary to a coding region of the mRNA. Support for this claim is found at page 19 lines 12-15 where such a oligonucleotide is described for FSH mRNA. Claims 66-68 recite specific lengths for the oligonucleotides and are supported by original claims 21 and 22 reciting at least 14 bases and about 23 bases respectively. Claims 69 and 70 recite viral and hormonal mRNA. Support for these target molecules is found in the specification at page 4 lines 1-3. Support for the claim 71 is found in the specification at page 5, lines 24-27 and in original claim 29. Support for claim 71 reciting an oligodeoxynucleotide is found in pending claim 53.

REJECTIONS:

This application is a Rule 62 file wrapper continuation of USSN 07/633,452. In the previous application, the Examiner raised a *prima facie* obviousness rejection against claim 63. Claim 63 corresponds in scope to new claim 64. In the event that the rejection is maintained over the pending claims, Applicant would ask that the Examiner consider and respond to the following remarks.

The prior art relied upon was Itakura *et al.* as a primary reference in view of Paterson *et al.* or Hastie *et al.* in view of Summerton or Miller *et al.*

Itakura is relied upon for disclosing means to synthesize oligonucleotides.

Secondary references of Patterson and Hastie teach hybrid arrested translation.

Tertiary references, Summerton and Miller were relied upon for disclosing uptake of oligonucleotides by cells. Miller is further cited for teaching nonspecific inhibition of cellular protein by binding oligoribonucleotides to cellular mRNA and for suggesting that increased length would result in increased specificity.

A. The law.

The Applicant respectfully urges that the *prima facie* case of obviousness is not properly set forth by the combination. A legally proper *prima facie* case of obviousness consists of three separate and distinct tests. The examiner must first identify the salient elements of the claimed invention in the prior art. Secondly, the examiner must identify an express suggestion or articulate an objective reason motivating the claimed combination of elements. Finally, there must be an objective reason for one of skill to conclude that the combination would function successfully. If any of the three tests are not met the *prima facie* case of obviousness is not set forth.

In addition, the examiner bears the initial burden of setting forth the three tests in a proper manner. Once set forth, the applicant may chose to rebut or traverse the rejection. Rebuttal is the presentation of argument or facts that challenges the sufficiency of the three tests setting forth the rejection. Traversal presumes the propriety of the *prima facie* case of obviousness and attempts to overcome the rejection by a showing of unexpected advantages.

Applicant will rebut the *prima facie* case of obviousness and respectfully reminds the Examiner of three basic rules applicable to rebuttal arguments. First the factual information presented in the rebuttal argument must not be considered for its ability to rebut the objective rationale of the extant

rejection. The Examiner must collect all the facts both pro and con and consider them collectively.[1] *Ex parte Ohsaka*, 2 USPQ 2d 1461, 1462 (PTOBPA&I 1987).

The second rule is that the examiner must consider all the rebuttal arguments and cannot ignore teachings away simply because they contradict the factual underpinnings of the obviousness rejection. *Application of Lunsford*, 148 USPQ 721, 724 (CCPA 1966).

Finally, the third rule is that the applicant does not need to establish that the examiner's *prima facie* case is wrong by a preponderance of the evidence. The applicant need only rebut the element at issue such that its resolution is in equipoise. An issue in equipoise is not determinable in light of the facts. In other words, if the examiner relies on facts, which unrebutted, establish by a preponderance of the evidence that motivation or expectation of success is present, applicant need only present evidence in rebuttal that is sufficient to place the issue of motivation or expectation of success into equipoise. *In re Oetiker*, 24 USPQ 2d 1443 at 1447 (Fed. Cir. 1992).[2]

Having articulated the rules for setting forth a legally sufficient *prima facie* case of obviousness, Applicant respectfully urges that the claims are patentable over the prior art. For some claims, the salient elements are not recited by the prior art. For other claims, the motivation is inadequate, and for others there is no reason for one of skill to have predicted the success of the invention based upon the prior art.

B. Based upon the prior art, one of skill would not have a reasonable expectation that the invention would work.

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[1] The Patent Board of Appeals, citing the Federal Circuit in In re Piasecki (223 USPQ 785, 788, 1984), stated in Ex parte Ohsaka: "when prima facie obviousness has been established and evidence is submitted in rebuttal, the decision-maker must start over...An earlier decision should not, as it was here, be considered as set in concrete...[T]he examiner must consider all the evidence anew. 2 USPQ 2d 1461, 1462 (PTOBPA&I 1987).

[2] Judge Plager in his concurrence in *In re Oetiker*, 24 USPQ 2d 1443 at 1447 (Fed. Cir. 1992) stated:
Specifically, when obviousness is at issue, the examiner has the burden of persuasion and therefore the initial burden of production. Satisfying the initial burden of production and thus initially the burden of persuasion, constitutes the so-called prima facie showing. Once that burden is met, the applicant has the burden of production to demonstrate that the examiner's preliminary determination is incorrect. ... If, as a matter of law, the issue is in equipoise, the applicant is entitled to the patent. [emphasis added].

The Federal Circuit articulated this essential feature of a proper *prima facie* case of obviousness in *In re O'Farrell*, 7 USPQ 2nd 1673 (Fed Cir. 1988). The Federal Circuit stated: "For obviousness under §103, all that is required is a reasonable expectation of success." Applicant urges that the prior art did not establish a "reasonable expectation of success". Prior to his invention, Applicant believes that there was no expectation of success - only a hope that the invention might work. At most, the prior art presents a classic example of an experiment that was "obvious to try." Such prior art cannot properly support a *prima facie* case of obviousness.

Furthermore, if the Examiner is of the opinion that the prior art went beyond a naked hope, Applicant urges that even when read in its most favorable light, the expectation is in equipoise. One of skill could not conclude one way or the other if the invention would be successful.

Miller is a primary reference suggesting that oligonucleotides might inhibit cellular protein synthesis. The authors indicate that their work suggests such a possibility. The two most suggestive textual statements have been cited by the Examiner. The abstract states:

> Our biochemical studies suggest that inhibition of cellular protein synthesis might be expected if $G^mp(Et)G^mp(Et)U$, $G^mp(Et)G^mpU$, and G^mpG^mpU, which have been taken up by or formed within the cell, physically bind to tRNA and mRNA and inhibit the function of these nucleic acids.

The last paragraph states:

> The triester $G^mp(Et)G^mp(Et)U$ is complementary to target nucleic acids involved in cellular protein synthesis and has a transitory effect on the cells which is then relieved by its degradation. Such a transitory effect of inhibition may provide a convenient and useful way of imposing a temporary interruption of cellular functions. On the other hand, neutral oligonucleotide analogues with longer chain lengths and a greater resistance to hydrolysis would be expected to achieve a greater specificity and effectiveness in regulating the function of target nucleic acids inside the cell over a long period of time. The observations of the present study lay the groundwork for continued investigation into the use of neutral oligonucleotide analogues as proves and regulators of nucleic acid function within living cells.

Unlike the reference in the *O'Farrell* decision, Miller does not state with positive *affirmance* that the concept of using nucleic acid analogues will regulate the function of a "target" nucleic acid within a class of nucleic acid types. The suggestion by Miller is clearly ambiguous. The authors' work is directed to general inhibition of all protein expression by using a trimer to bind to the amino acid accepting codon of a tRNA. There is no evidence of binding to target mRNA or of any inhibition of protein expression due to trimer binding to mRNA. The word "specificity" is ambiguous as to whether the authors mean longer oligonucleotides that do not bind to noncritical regions of the tRNA and that are specific for the amino acid accepting codon of tRNA or, whether the longer oligonucleotides will bind specifically to the initiation region of a mRNA, or as the Examiner would read the passage, that a longer oligonucleotide would bind with greater specificity to the coding region of an mRNA.

Even if one were to ignore the ambiguity in the text of Miller and read it in a light least favorable to applicant, the authors only state that their work *may* provide grounds to justify further inquiry towards proving the viability of the theory. A suggestion to continue investigation does not equate to a suggestion that there is a reasonable expectation that the concept is functional, and therefore, Miller fails to meet the test articulated by the *O'Farrell* decision.[3] A reference merely speculating as to what might be possible cannot be elevated by the Patent Office to the level of a patent defeating prior art reference. This is old law dating back to the *Barbed Wire Patent* decision, *Washburn & M. Mfg. Co. v. Beat 'Em All Barbed Wire Co.* 143 US 275 (U.S.S. Ct., 1894). Modernly this law has been followed in the cases where *in vitro* evidence of therapeutic utility was suggestive of *in vivo* pharmaceutical uses with the courts finding that suggestions in unpredictable arts are not sufficient to set forth the *prima facie* case of obviousness. See for example, *In re Carroll*, 202 USPQ 571 (CCPA 1979) and *In re Gangadharam*, 13 USPQ 2d 1568, (Fed. Cir. 1989 unpublished citation).

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[3] See also *In re Dow*, 5 USPQ 1529, at 1531 (1988) where the Federal Circuit stated:
The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art. [citations omitted]. Both the suggestion and the expectation of success must be founded in the prior art not in the applicant's disclosure.

The Miller reference alone is clearly insufficient to raise a reasonable expectation that binding complementary oligonucleotides to the coding region of mRNA would arrest translation. In addition, Applicant fails to see any text in the other references relied upon by the Examiner which would supply the requisite expectation. The Applicant respectfully asks the Examiner to either identify the text of the other references which alone or in combination provide the element of expectation required by the *O'Farrell* decision.

If the Examiner remains of the opinion that Miller set forth a reasonable expectation that the oligonucleotides will regulate expression of a specific mRNA, Applicants ask that he consider the following five objective reasons contradicting the suggestion of Miller read alone or in combination with the other references.

1. The Secondary structure of the mRNA made it an unlikely target for control of expression by complementary oligonucleotides.

This invention goes against the conventional wisdom of the time. The conventional wisdom, as articulated in the prior art and later references discussing the state of the art in 1981, was that the secondary structure of mRNA is so extensive that there was no reasonable likelihood that a oligonucleotide complementary to a coding sequence would have sufficient access to arrest translation. In addition, those of skill understood that the mechanism of peptide elongation by ribosomes involved the destabilization of the extensive secondary structure of the mRNA. For this reason, the notion of hybridizing a complementary oligonucleotide to a coding region was contrary to conventional logic. If the ribosomes could read a coding region that was naturally constrained by extensive secondary structure, it was not likely that the hybridization of a complementary oligonucleotide would arrest translation. The ribosome would simply "toss" the duplexing structure aside.

Although there is little express language in the prior art articulating the above concerns, the literature indirectly compels one to conclude that secondary structure and ribosomal helix destabilization were concepts teaching away from the

invention. References published after the priority date of the subject application do expressly identify the state of the art at the time of the invention.

The extensive secondary structure of mRNA was well understood in 1981. In the book, The Ribonucleic Acids, 1977, Eds. Stewart and Latham, Springer Verlag, Chpt. 4, "Messenger RNA" by J.M. Adams, the extensive secondary structure in phage mRNA and eukaryotic mRNA is described. Those of skill recognized the importance of secondary structure for its absence was critical for initiation of ribosome binding. W. Salser, in his chapter Globin mRNA Sequences: Analysis of Base Pairing and Evolutionary Implications, in *Chromatin* Vol XLII (1978) pages 985-1001 at page 992, provided a graphic illustration of the extensive amount of secondary structure in the typical mRNA. In Dr. Salser's illustration, the sequences involved in ribosome initiation and interaction are dramatically free of secondary structure. Dr. Adams, on page 103 in his section (e) entitled "Influence of secondary and tertiary structure on initiation," also discusses the importance of secondary structure on initiation of translation. In chapter 7, by Wettenhall and Clarke-Walker of *The Ribonucleic Acids*, the authors on page 258 in a section entitled "Messenger RNA structure" expressly state, "[t]he role of mRNA structure in initiation reactions is receiving considerable attention." These authors also discuss the role of secondary structure of mRNA on translation efficiency.

The concern over availability of the coding region to bind oligonucleotides is made more apparent when one looks at the secondary references relied upon by the Examiner. Both Paterson *et al.* and Hastie *et al.* used denaturing conditions to relax the secondary structure of their mRNA prior to hybridizing nucleic acids. For example, Patterson used 100°C for 30 seconds and Hastie used temperatures between 45° and 65°C. When Hastie wanted to slow down hybridization, they used gentle heating (45°C) but relatively high salt concentrations of 0.18 M.

A number of other references expressly stated that the targeting of a coding regions would not be a preferred target for a oligonucleotide agent expected to control expression. Pluskal *et al. Biochem. Soc. Trans.* 7:1091-1093 (1979),

wrote that their work with a heterogenous mixture of low molecular weight oligonucleotides non-specifically inhibited translation, but that the mode of action was not affected by preincubation with the mRNA. One of skill is left to conclude that the translation inhibition observed by Pluskal did not occur via complementary binding interactions between the oligonucleotides and the mRNA.

Continuing in temporal order, the Ts'o patent further suggests away from the Applicant's invention. While the Ts'o patent is not effective prior art, Applicant submits that its teaching is representative of the thoughts of those of ordinary skill. In the Ts'o patent, the claimed oligonucleotide analogues are described as inhibiting expression of a preselected sequence, either a cellular nucleic acid or a viral nucleic acid. Ts'o uses trimer analogues and at column 25, lines 5-10, they state that their analogues inhibited poly U messages but not globin messages. In column 26, lines 5-34, Ts'o explains that they saw no inhibition *in vivo* either bacteria or hamster cells.

Ts'o's results are consistent with later reports. These later reports actually explain what was intuitively obvious to those of ordinary skill at the time Dr. Tullis filed his application. There was no reason or basis to conclude that the coding region of mRNA was an effective target for *in vivo* control of expression. There was no evidence that the trimers used by Ts'o inhibited elongation on a mRNA either *in vitro* or *in vivo*. Either the coding regions of mRNA were physically unavailable to complementary oligonucleotides or even if binding to the coding regions, the oligonucleotides were unable to arrest the elongation process of the ribosomes. The latter concept is particularly compelling because to elongate, ribosomes have to untwist the secondary structure of mRNA.

Perhaps the most express and compelling articulation of the true state of the art from 1977 until after the effective filing date of the subject application is found in Dr. Miller's own work published in two parallel reports in 1985. In Blake, Murakami and Miller, *Biochemistry 24*, 6132-6138 and *24*, 6139-6145, the authors discuss at length their concerns over secondary structure on the availability of subsequences of mRNA to complementary oligonucleotides. In the first of the two companion articles (A22), Dr. Miller looked at 8-12 mer

oligonucleotides and concluded on page 6135, that in the cell free rabbit reticulocyte protein expression system, 8 mer oligonucleotides do not arrest protein elongation, but that if bound to the initiation codon region the oligonucleotides will arrest translation. On the second column of page 6136 , Dr. Miller in summarizing the state of the art expressly states:

> The above results are in agreement with the recent findings of Liebhaber *et al.* (1984). They found that cDNAs to human globin mRNA which cover the initiation codon or extend into the 5'-noncoding region are able to completely inhibit translation in a rabbit reticulocyte lysate. In contrast cDNAs which cover only the coding region exclusive of the initiation site are not effective at blocking translation. These authors postulate that a helix-destabilizing activity associated with the reticulocyte ribosomes is able to disrupt secondary structure during the elongation step but not the initiation step of translation. **Thus, cDNAs or oligonucleotides bound to the coding region of mRNA would be expected to be unable to prevent translation in the reticulocyte system.** [Emphasis added]

Dr. Miller is summarizing two points to explain why oligonucleotides binding to the initiation codons will inhibit translation but those binding to coding regions will not inhibit translation. He first explains that the ribosomes will simply remove the oligonucleotides bound to the translated region (page 6136, column 2), and second, he explains that the secondary structure of mRNA precludes the binding of the oligonucleotides to the translated region (page 6137). He implies this is true even for cDNAs which may be hundreds of bases long.

In the second article, Dr. Miller is again reporting on the ability of oligonucleotides to arrest *in vitro* translation. In this report the oligonucleotides are between 6 and 11 bases long. The authors are reporting some degree of success with the binding of oligonucleotides to mRNA coding regions. Although the success of the method is being reported, the authors are clearly articulating the concerns over secondary structure which Applicant urges was the conventional wisdom in 1981. For example at page 6144, the authors state:

> In addition to the effect of mRNA secondary structure, the region of the mRNA to which the oligonucleotide methylphosphonates binds influences its effectiveness as an inhibitor of mRNA translation. The results in Table II suggest that oligomers complementary to the 5' end

> and initiation codon regions are somewhat better inhibitors than the oligomers which bind to the coding regions. **Thus it appears that the initiation step of translation is more sensitive to oligomer mRNA binding than the elongation step of translation.** [Emphasis added]

Finally, as late as 1986, the literature is suggesting that short oligonucleotides will only arrest translation if bound to the initiation sites. In particular, Haeuptle *et al.* using a cell free translation system, clearly states that size of the oligonucleotides bound to coding regions is a critical key to ensuring arrest of translation. Just as is taught by the subject application with its 1981 effective filing date, Haeuptle reports in 1986 that the oligonucleotides must be 10 bases or greater in length before substantial inhibition of translation is demonstrated. The Examiner should also note that the authors, in deference to secondary structure, are using 55°C for 5 minutes to relax the mRNA sufficiently to allow the oligonucleotides to bind to their target regions.

In conclusion, Applicant submits that the conventional wisdom in the art, at the time of the invention, taught away from his invention. The literature cited above is offered as evidence of this wisdom. This literature clearly teaches that the secondary structure of mRNA, especially in the coding region, was thought by those of skill in 1981 to be extensive and that this secondary structure would preclude binding of a complementary oligonucleotide to the coding region mRNA for the purpose of arresting translation. Also, there was reason to believe that the helix destabilization of ribosomes during elongation would have easily displaced any oligonucleotide duplexed on the coding region. Therefore, one of skill would not have considered the claimed invention to have a reasonable expectation of success in 1981 when Dr. Tullis originally filed the subject patent application.

2. Even if the prior art oligonucleotides of Miller were binding to the coding region of an mRNA, they could not have arrested translation.

Although the Miller (1977) reference suggested that the mRNA was an equivalent target to the tRNA, in reality the tRNA was the actual target controlling expression. Miller uses only trimer oligonucleotides and suggests longer

species but does not specify how much longer the oligonucleotides need to be. In fact, the Miller trimers were not useful as inhibitors of translation even if bound to a coding region of an mRNA.

The Examiner is directed to the 1986 reference of Haeuptle *et al.* Haeuptle describes the *in vitro* arrest of translation by oligodeoxynucleotides. The authors relaxed the secondary structure of mRNA encoding lysozyme using 55 C and hybridized oligonucleotides of varying lengths to a portion of the coding region. On page 1435 and in figure 4 on page 1439, they present evidence of their inability to arrest translation using short oligonucleotides of 5 mer. The 5 mer species are simply too small to have sufficient binding strength to arrest the progress of a ribosome. The authors reported successful arrest of translation with their 10, 15 and 20 mer species.

The Miller reference should not be interpreted beyond the scope one of ordinary skill would have interpreted the text in 1977. Clearly those of ordinary skill would not have believed that trimers or undefined longer species would have had the ability to arrest translation based on a study which obviously targeted the initiation regions of tRNA as binding sites. These regions have little or no predictable secondary structure. As explained above, the extensive secondary structure of mRNA was a primary reason why those of skill would have avoided the coding region of mRNA to arrest translation. To argue that in 1981, one of ordinary skill would have predicted *in vivo* utility for oligonucleotide binding to the translated portions of mRNA is to read Miller in a vacuum, and ignores the authors' data and the general understanding of the accessibility of the coding region of mRNA due to secondary structure.

Having explained that the Miller reference could not have suggested to one of skill that their results suggested the *in vivo* uses claimed by the Applicant, it is submitted that the rejection should be reconsidered and withdrawn.

3. The Examiner has read the Miller reference in light of the Applicant's work and applied hindsight to his interpretation of the reference.

There are two further reasons why Miller fails to suggest the claimed invention. First, the reference fails to expressly identify the coding region as the target region for arresting oligonucleotides, and secondly, it is silent on the appropriate length suitable for arresting translation. Consequently, Applicant respectfully urges that absent the subject disclosure, one of skill would not have read Miller's text in 1977 to 1981 and given that text the same interpretation used by the Examiner to reject the claims of this application. This is a typical and improper use of hindsight to support a *prima facie* case of obviousness.

When read by itself, and in context of 1981, the Miller reference is silent as to the target sequences on a mRNA to which the oligonucleotides would bind. Messenger RNAs have several functional domains. The coding region is only one domain. These various domains are described as "signals" by W. Salser in his chapter Globin mRNA Sequences: Analysis of Base Pairing and Evolutionary Implications, in *Chromatin* Vol XLII (1978) pages 985-1001. The signal sequences are described on page 988 as including "ribosome binding, mRNA processing, transport from the nucleus to the cytoplasm and so on".[4] Having explained that there are various target domains on a mRNA and that the Miller reference fails to identify a specific domain as a target for oligonucleotides, the Applicant urges that the Examiner has applied the teachings of the subject application to interpret the Miller reference beyond the scope one of skill would have given the reference in 1981.

Secondly, the Applicant also notes that the Miller reference refers to oligonucleotides longer than their triesters. The authors do not specify how long a oligonucleotide they would use. In contrast, the Applicant clearly teaches that the length of the oligonucleotide is an important feature of the invention. This was later confirmed by Haeuptle in 1986, in which the authors report that the only oligonucleotides suitable for arresting translation are 10 mer or longer, and by

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[4] It was even reported in 1977 that low molecular weight oligonucleotides would interfere with protein translation by binding with the polyadenylation tail. See Pluskal *et al.* 583rd Meeting, Cambridge Vol 7:1091-1093 at page 1091.

Blake (A22, 1985), which reported that 8-mer were unable to arrest translation when bound to the coding region.

Applicant respectfully urges that Miller was referring to longer oligonucleotides which bind to regions of the mRNA free of secondary structure and not to the coding regions. In Dr. Salser's review article of 1978, he includes a figure 3 depicting the proposed secondary structure of a mRNA. It is an incredibly complex and twisted structure. Only a few regions are accessible to a complementary oligonucleotide and these are designated as points of initiation and tRNA interactions. These open regions which are generally found as single-strand hairpin loops are longer than 3 mer and thus Applicant urges that the most reasonable interpretation of Miller text is that these regions are the proposed targets.

For the above reasons, Applicant urges that Miller's reference to "greater specificity" was never intended to be a suggestion for controlling the expression of particular "target" proteins, but a suggestion to use longer oligonucleotides to bind **specifically** to the open regions of the mRNA that bind to the ribosomes. When placed in its proper context in view the literature, both prior art and latter art, one of skill would conclude that the Miller authors are referring to the use of oligonucleotides that bind to regions of the mRNA that are open and not bound up in extensive secondary structures. Such regions exclude the coding regions.

4. The Examiner has misinterpreted the use of "specificity" by Miller in 1977.

The Examiner makes much of the use of the final paragraph of Miller, 1977 stating: "... oligonucleotide analogues with longer chain lengths and a greater resistance to hydrolysis would be expected to achieve a greater *specificity* and effectiveness in regulating the function of target nucleic acids...". The Examiner reads this sentence to include oligonucleotides binding to the coding regions of mRNA. Applicant has compelling evidence that the authors' reference to specificity was in a general context and did not refer to the coding regions of

mRNA encoding specific proteins. Instead, the authors were referring to the amino acid accepting end of the tRNA and the initiation sites of mRNA.

The Applicant asks that the Examiner take notice of the Dr. Miller's later publication, Blake *et al.* (1985A). There on page 6137, column 2 is the word "specific[5]" used in an identical context to the word "specificity[6]" used in 1977. Miller is again asserting that his work suggests *specific* control of expression by binding oligonucleotides to mRNA. But the context of the later reference clearly implies that the oligonucleotides are not directed to the coding regions of the mRNA. The Blake article states that the only available regions are the initiation points due to secondary structure. For the Examiner to continue to interpret the text of Miller 1977 in an unlimited and broad context including the coding regions of mRNA is to ignore the scientific realities of the contemporaneous literature.

Miller in 1977 understood that the secondary structure of the mRNA would render the coding region unavailable to complementary oligonucleotides. His contemporaries certainly took the mRNA secondary structure into account. As explained above, both Paterson and Hastie used high temperatures to first denature their mRNA and then hybridize oligonucleotides. For one to conclude that Miller's language in 1977 refers to the coding regions of mRNA is to read beyond the interpretation one of ordinary skill would have given such language in 1977.

5. In vivo arrest by complementary oligonucleotides is not predictable from the cited in vitro studies.

With exception of the Miller reference, the prior art relied upon by the Examiner involves *in vitro* studies. By themselves these references do not set forth the *prima facie* case of obviousness. Although the Examiner is not arguing that the references are sufficient absent Miller, Applicant would like to point out that the cell free systems of Hastie and Patterson are not predictive of the conditions inside

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[5] Dr. Miller states in Blake *et al.* at page 6137, column 2, The results of this study show that sequence-specific oligodeoxynucleotides can be used to arrest translation of specific mRNAs in a selective manner in cell-free systems.

[6] At page 1995, Miller states: "neutral oligonucleotide analogues with longer chain lengths and a greater resistance to hydrolysis would be expected to achieve a greater specificity and effectiveness in regulating the function of target nucleic acids inside the cell over a long time period.

the cells. The osmotic potentials, the salt and pH conditions, the microenvironments within the various compartments of the cells, the secondary structure of the mRNA *in vivo*, the physical pressure and gel like consistency of the cytosol, all preclude the ability of one of skill to predict with a reasonable certainty that complementary oligonucleotides could bind with sufficient strength if at all to the coding regions of mRNA to selectively arrest translation. As Dr. W. Salser succinctly stated in his review article of 1978, "The problem is then to assess which parts of the structure in Figure 3 [mRNA] may actually exist in the complex milieu of the cell."

Furthermore, even if one were to ignore the conventional wisdom that the secondary structure of mRNA rendered the coding region inaccessible to complementary oligonucleotides, there were a number of unknown parameters which alone or in combination render the question of successful regulation of expression by the claimed methods unpredictable. The mRNA of eukaryotes is produced in membrane bound nuclei. The art demonstrated that oligonucleotides would be taken up by cells, but not whether the oligonucleotides would be allowed access to the internal regions of the nuclei. Befort *et al.*, at page 184, states that exogenous oligonucleotides used as antiviral agents enter cells but are primarily bound to the microsomal fractions and do not significantly enter the host cell nuclei. It was known that messenger ribonucleic acid proteins bound to mRNA. According to Pain and Clemens, in *Comprehensive Biochemistry*, Vol 19B, Part 1 at pages 14-15 (1980), the function of these proteins was unknown. These proteins might have played a role in the storage and delivery of the mRNA to the correct ribosomes (membrane bound or not) and might have interfered with the access of the mRNA complementary oligonucleotides. As the authors go on to explain, only 10% of the total mRNA produced by the cells is actually used by the ribosomes to produce protein. That unknown mechanism, perhaps controlled by messenger ribonucleoprotein, could very well have rendered the mRNA unaccessible complementary oligonucleotides.

Finally, even if (a) the mRNA were physically accessible complementary oligonucleotides, (b) issues of secondary structure were ignored,

(c) the fact that short oligonucleotides might not have sufficient binding strength to block a ribosome designed to untangling internal duplexes in mRNA were ignored, (d) the fact that the use of long complementary oligonucleotides would mimic mRNA and likely have their own secondary structure to interfere with hybridization of mRNA, and (e) it was not understood that the majority of mRNA are not actually translated by cells but rapidly turned over - the flooding of a living cell with oligonucleotides at the concentrations necessary to effectively bind to mRNA might have been toxic in a non-specific way. Nucleic acids are notorious for binding divalent cations. These cations are needed for homeostasis. The presence of an abundance of divalent cation scavengers could have wreaked havoc on the cells' electropotential and rendered *in vivo* uses as claimed inoperable.

C. Claims 66-68 recite elements not found in the prior art.

Applicant urges that even if the above arguments are unconvincing to the Examiner, Claims 66-68 are separately patentable. These claims recite oligonucleotides of specific sequences and lengths. These lengths are a salient part of the claims and are not recited by any of the prior art references. The oligonucleotides of the closest prior art are only three bases long. As explained in Haeuptle, oligonucleotides of such length are not only nonspecific for any one protein, they are unable to arrest the elongation process of translation.

To properly set forth a *prima facie* case of obviousness the Examiner must find the salient elements in the prior art. The Examiner is reminded of Judge Rich's opinion in *Hybritech Inc. v. Monoclonal Antibodies, Inc.* 231 USPQ 81 (Fed. Cir. 1986) where the patentability of the use of monoclonal antibodies in a conventional sandwich assay hinged on the claims recitation of an affinity constant of 10^{-8}. Although the polyclonal antibodies of the prior art inherently used such antibodies, the prior art did not disclose the preferred affinity constant. Likewise in this situation, the prior art is silent on these elements of claims 66-68.

In view of the above remarks, Applicant believes the claims are in condition for allowance. If the Examiner is of the opinion that a telephone conversation would expedite prosecution, he is invited to call the undersigned attorney at the number provided.

Respectfully submitted,

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